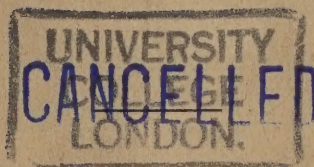


*Dr. D. V. Hall
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of John J. Abel*

ON POISONS AND DISEASE AND SOME EXPERIMENTS
WITH THE TOXIN OF THE BACILLUS TETANI.

JOHN J. ABEL

EMERITUS PROFESSOR OF PHARMACOLOGY AND DIRECTOR OF THE LABORATORY FOR
ENDOCRINE RESEARCH, THE JOHNS HOPKINS UNIVERSITY



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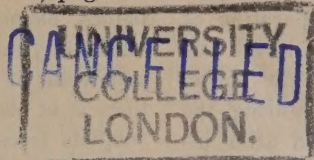
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ON POISONS AND DISEASE AND SOME EXPERIMENTS WITH THE TOXIN OF THE BACILLUS TETANI^{1, 2}

By Dr. JOHN J. ABEL

EMERITUS PROFESSOR OF PHARMACOLOGY AND DIRECTOR, LABORATORY FOR ENDOCRINE RESEARCH,
THE JOHNS HOPKINS UNIVERSITY

SECTION N of our association has, as you are aware, the very wide field of the medical sciences for its subject-matter, and it seems appropriate for a member of that section to address you on a subject which, while a primary concern of medicine and biology, is yet not without interest to scientists in general. I am therefore presenting to-night some considerations on poisons and disease.

Many of us who are not students of the medical sciences are but little interested in theories of disease. When illness overtakes us we wish to be cured "safely, quickly and pleasantly." There lived in the first century B.C. in Rome a Greek, a very successful and learned physician who was also a writer and philosopher, who promised his patients to cure their diseases according to that excellent and oft-quoted formula, "*Curare tuto, celeriter et jucunde.*" We may be sure that this Greek scholar, the friend of the great writers and statesmen of his day, was a bit of a humbug as a physician, for only the God of healing himself could always make such a promise good. Nevertheless, the physicians of our day frequently succeed in curing certain diseases in accordance with this happy formula, but their greatest achievement in modern times is the prevention of disease.

Medicine in its cruder forms was no doubt, next to food, clothing and shelter, one of the earliest concerns of primitive man. He must soon have learned that certain bulbs, roots, seeds, berries, leaves and other parts of plants could not be eaten without fatal results; and he also soon learned that the bites of venomous serpents led to a more or less rapid death, and that there were numerous poisonous insects and grubs, poisonous fish, and other creatures on land and in the waters that had to be guarded against and many of which could not be eaten with impunity. We may be certain also that primitive man very early

acquired a knowledge of how poisons that destroyed him could be utilized in the chase to bring down large and dangerous animals that were used by him for food and other purposes. The removal of venom from the poison sacs or the crushed head of a deadly snake and its application to the head of an arrow or a spear is an act that requires only the most elementary degree of mental acumen. As far as we know, every race of man in its early stages made use of arrow poisons, and the practise still prevails among uncivilized peoples to-day. The number of poisons that have been and are still used for this purpose, according to students of the subject, probably runs into the hundreds. We know that poisons suitable for this purpose are found in large number among plant products and in innumerable forms of animal life from the lowest to the highest. They range in fact from the most nauseating of odoriferous, volatile, but often only relatively harmful compounds to the most lethal of the fixed venoms and toxins. The invention of smearing arrows, spear heads and other weapons with poisons was certainly of the highest utility for primitive man in the chase, as it enabled him to kill the largest and most dangerous animals with the least amount of danger to himself. The antiquity of the invention can be traced back to a very early stage in man's history, and it is probably the first example of a physiological or pharmacological experiment made by him. The experimental pharmacology, the surgery and medicine of primitive man were early burdened with a belief in the supernatural causes of disease, and such rational experimentation as there was gave place to "a nightmare of evil spirits," magic and sorcery and a reliance on enchantments, as is still the custom in our day among backward peoples. Stinging insects and venomous serpents were no doubt the first among animal forms to invent hypodermic injection, a procedure which was introduced into medical practise only in the years 1845 to 1856. The venoms of stinging insects and deadly serpents are products of their own metabolism, formed in special glands, but it must not be thought that they have been developed in the long course of time solely for self-protection. It is well known to

¹ Address of the president of the American Association for the Advancement of Science, Boston, December 27, 1933.

² A grant from the Carnegie Corporation of New York enabled my coworkers and myself to carry out the experimental work described in the second part of this address.

workers in institutes devoted to the preparation of antivenins that the venom is indispensable to the serpent for the maintenance of its health. The poison can not be expressed from the glands during the period of sloughing the skin or too soon after ingestion of food without fatal results to the reptiles. The venoms, therefore, play an essential rôle in the internal economy of these creatures, perhaps quite aside from the fact that they contain proteolytic ferments essential for digestion. Similarly, the poisons of bees and wasps appear to aid in the development of their eggs, after they have been fixed on them.

The mortality due to venomous snakes and stinging insects is a trifling matter compared with that due to the biting insects. These also introduce beneath the skin small amounts of irritating substances, that are of negligible importance in comparison with the extrinsic or adventitious agents that may be introduced into the bite at the same time. The biting insects, such as certain kinds of mosquitoes, fleas, the tsetse fly, the African horse fly, the body louse, or "cootie," and various ticks, are responsible for the great scourges that have decimated mankind and destroyed his domestic animals. Their responsibility is, however, of an indirect character, as they merely happen to be the carriers of deadly varieties of microorganisms. Among the serious diseases due to biting insects are malaria, yellow fever, black death, or bubonic plague, African sleeping sickness of man and the allied diseases of domestic animals, typhus fever and many more.

The elucidation of the causes of these and other infectious diseases, the development of specific curative agents for some of them and methods for their eradication and prevention are among the great achievements of the biological and medical sciences during the past sixty years. Only the most highly civilized and wealthy nations of the world are in a position to suppress more or less completely the dreaded insect-borne diseases of which I have been speaking. Malaria is one of them that can be completely eradicated, and yet Professor Bass writes of this disease as follows: "Unfortunately the outlook is not so favorable for the less civilized and less fortunately situated people of the world. The proportion of the people in many countries who have the disease varies from none in some countries to practically 100 per cent. in others. Even now millions, yes, more than a hundred million people throughout the world, suffer with it every year and more than a million die."

Among the remains in caves of the Madelenian epoch in the Vézère, the Dordogne and elsewhere in France are found the bones of human beings and those of many large animals, as the bear, the aurochs, mammoth, reindeer and others of that time. The man of this period fashioned for himself from bones spear

points, harpoons, hooks, needles and other implements, and carved pictures, on bones, reindeer antlers and teeth, of many of the animals of his time, and there is at least one considerable work of his art that "depicts trees, horses and an aurochs, and a snake biting a man's leg." In this remote period of antiquity the serpent, as ever since, "bruised the heel of man." What is of especial interest to us is the fact that the spear heads and harpoons of the man of that epoch had a series of pits on them or a series of parallel grooves, which some archeologists believe were designed to hold deadly poisons. It is apparent that such poisoned weapons were also effective in warfare, and this use of poisoned spears and arrows by primitive man against his human enemies has never been forgotten. South American Indians invented "poison gas" long ago, according to Baron Nordenskiöld, who says: "The Indians of (South) America cultivate a spice plant, Spanish pepper (Cayenne pepper), which is of the greatest importance to them. They have discovered that by the burning of this pepper there is developed a sort of "poison gas" that proves effective in laying siege to villages fortified by palisades." The Romans in their wars with the Gauls and Germans and other peoples in the early centuries of our era lost many of their men from wounds made by poisoned arrows—wounds that were of no significance of themselves. L. Lewin, an investigator and historian in this field of study, has gathered references from the literature that prove that the French, Swiss and Spaniards continued to employ poisoned weapons in hunting deer and the wild boar down to the fourteenth and even to the latter part of the sixteenth century, particularly in Spain. Some historian has said that war has been the chief industry of man throughout the ages and promises to continue to be so for many a long day. Certainly in our day war and preparations for war must still be counted among the greatest of our industries. At the moment chemists in the employ of governments everywhere throughout the world are busier than ever in inventing lethal gases, fluids and even relatively insoluble toxic substances for the destruction of the enemy's forces and, unavoidably at times, of his civilians.

We have mentioned arrow poisons that are secreted by the poison glands of venomous serpents. Such poisons are generally spoken of as venoms and sometimes as toxins, but it will be seen later that the latter term is generally applied in our day to the deadly poisons elaborated by pathogenic microorganisms. It is interesting to note that our words *toxic*, *toxin*, *intoxicate*, and the large number of combining forms and derivatives so widely employed in the biological sciences contain the root or stem of the Greek word for bow—*τόξον*—often used in the plural for arrows.

The poison for smearing arrows was originally termed *τοξικὸν φάρμακον*, but in the course of time the noun of the phrase was dropped and the adjective form, *τοξικόν*, "of, or pertaining to the bow," took on the significance, "poison for arrows," and then of poison in general. The literature of the ancients, as well as our own and that of other nations and their folk-lore, contain numerous figurative expressions based on this ancient use of poisoned weapons. Job mourns, "For the arrows of the Almighty are within me, the poison whereof drinketh up my spirit." Stockman, in writing of arrow poisons, after speaking of the widespread belief that malignant disease or pestilence is due to the invisible but deadly arrows of the offended gods, remarks "that even such an impalpable feeling as love was conveyed into the heart by means of Cupid's darts."

Many of you practise archery in your vacations, as a pastime or as an aid to health, and you may possibly know that the English scholar and man of letters, Roger Ascham, Latin secretary to Queens Mary and Elizabeth and the author of that well-known treatise entitled "The Scholemaster," wrote a book on archery in 1545, which he called, "Toxophilus," or Lover of the Bow. The treatise is in the form of a Socratic dialogue between Toxophilus, who defends the use of the bow as an indispensable national weapon and also as a means of dispelling the "grosse and colde humours" that "gather and vex scholars," and Philologus, who has to be convinced of the advantages derived from "shooting." The little work is no longer read in our day, but is full of classical learning and reflections on a variety of subjects touching the physical and moral health of men and nations. Through it the word "toxophilite" was introduced into the language, to designate the devotee of archery, and in England there was even a "Toxophilite Society" in the eighteenth century.

We have been speaking of the silently operating and, to primitive man, mysterious and deadly agents that are found so widely distributed in nature and that are usually designated as poisons, and we may ask, What is poison?

WHAT IS POISON?

Let me say to begin with that no one has ever been able to give a concise and accurate definition of a poison that would apply to every one of the many thousands of known poisons. All attempts to do this have simply ended in offering definitions that do not define. The medical jurisprudence of this country and of England has never attempted to define the word *poison*.

The exponents of the medical and biological sciences are, however, no worse off in respect to the impossibility of defining in a satisfactory manner such

terms as *living or life, disease and health*, than are the exponents of the other natural sciences in defining certain of their fundamental concepts.

Nature has not affixed a poison label to any particular substance or class of substances. The pharmacist does that. I have met intelligent people who actually believe that once a poison always a poison, and that it continues to exert its noxious power in some degree to the last molecule. If ten milligrams of nitrate of strychnine be dissolved in five hundred cubic centimeters of sterilized physiological saline solution, each cubic centimeter will contain 0.02 mg of the strychnine salt, but no one in the world could demonstrate either a harmful or a beneficial action after the injection of a half or one cubic centimeter of this solution underneath a person's skin or when so small a quantity as this is taken by mouth. We can, however, still detect the bitter taste of this highly diluted strychnine solution if we hold a cubic centimeter of it in the mouth for a few seconds, and if a wine-glassful were swallowed a mild stimulation of the digestive apparatus might or might not occur. The experience of physicians throughout the ages has convinced them that even the most deadly of poisons are entirely innocuous when given in certain minimal quantities. Scientists have arrived at the same conclusion on the basis of their experimental work. To be quite accurate, however, we can only say that neither a harmful nor a beneficial influence can be detected after such minimal doses by *any methods known to us at present*.

There is, however, another fact of fundamental importance for everything that is endowed with life, whether it be a plant or an animal or something that lies somewhere between the two. This fact may be expressed as follows: The harmful effects of a large dose of a drug or a poison do not fall off *pari passu* with the diminution in dose. The law of concomitant variation in respect to the two variables does not hold for the living cell. On the contrary, with the diminishing dose there may occur a turn-about in the action of the drug with the appearance of a highly beneficial action, one indeed that is indispensable for the continuance and maintenance of health, as is the case when we pass from the toxic dose of a hormone to its therapeutic dose. Examples of this beneficial action are seen daily in medical practise. Many powerfully acting drugs, to which the popular designation of poison is entirely applicable, such as strychnine, quinine, the digitalis glucosides, certain arsenicals, insulin, parathyroid extracts, the vitamins, and a long list of other drugs illustrate this principle when their dosage is restricted to a certain amount. This fact holds also for other agents of our environment that may be very harmful in their action when they come in contact with our tissues in too high a concentra-

tion. Sunlight and other forms of radiation are instances in point. In ordinary concentrations sunlight is beneficial, but it is deadly in its effect on many organisms when they are exposed to it for too long a time. Its scorching action on the skin of adults at high altitudes and in the tropics is well known, and it is also well known that very young children have died as a result of exposure for too long a time to the burning rays of the noonday sun without having been previously inured to its action.

POISONS IN OUR BODIES

An excellent illustration of our principle that toxic substances may be highly beneficial and even indispensable to life is furnished by certain poisons that are produced in our own bodies and circulate in our blood, at a concentration below the toxic level. Many will be surprised to learn that we ourselves are walking drug shops, and that an experienced chemist or pharmacologist would have no difficulty in preparing arrow poisons from some of our own organs that would have delighted the heart of primitive man. Let me name only a few of these autogenous poisons that serve not only to maintain our normal state of health but are also indispensable for the alleviation or removal of the symptoms of grave metabolic disorders—insulin, a hormone manufactured in certain cells of the pancreas, is one of them. It is known to all of you that when the concentration of this hormone in the blood falls below a certain level for any length of time, a serious disease known as diabetes mellitus inevitably appears. Our bodies probably require for the maintenance of health that the pancreas should produce and pass into the blood during each 24 hours about eight milligrams of this substance, and when, as I have just said, this amount is no longer furnished by the pancreas, diabetes results. You are also well aware of the fact that the subcutaneous administration of insulin as manufactured from the pancreas of beees causes an immediate and spectacular improvement in the diabetic patient. As long as the deficiency of insulin persists, the patient must have his daily injections of the insulin from animal sources. During the first years of this so-called replacement therapy, it was occasionally observed that children fell into convulsions after the administration of an overdose of insulin. This extraordinary effect is daily seen in the laboratories in which insulin is being standardized for medical practise by testing it out on rabbits that have been previously kept without food for 24 hours. After a certain dose these animals fall into the most violent epileptiform convulsions, which generally end fatally if nothing is done, but which can be immediately and entirely dispelled by the intravenous injection of a little glucose. After the injection of this antidote the animal is immedi-

ately restored to perfect health. A similar treatment was applied to children that had been given too much of the drug. It is the function of this hormone to supervise and control, so to speak, the metabolism of carbohydrates in our tissues, and it is especially toxic in overdose for those animals or human beings the sugar content of whose blood happens to be low at the time of its administration for curative purposes. No experimentalist can doubt that an intravenous injection of 20 or 30 mgm of this hormone would suffice to kill a person of average weight who has gone without food for a couple of days. It is well known to physicians that under certain circumstances, as when a tumorous growth invades the hormone-producing organ, or even without this cause, an excessive amount of the hormone is produced and distributed in the body. In such instances serious impairment of health and death may occur. Fortunately, indeed, we are not often poisoned in this manner. In one recently reported instance a year-old child had repeated epileptiform convulsions, with retarded mental development and only about half of the normal amount of sugar in the blood, in consequence of an overproduction of insulin in its pancreas. Removal of a large part of the overactive pancreas caused the child's condition to return to the normal state. Cases of this nature have also been seen in adults, and here also an operative procedure was followed in some instances by an improvement in health. Conditions of this type, in which the causative agent is produced in insufficient amount or in excess in our own bodies, are spoken of as diseases of endogenous origin. Another, and one of the most striking instances of overproduction of a hormone, is seen when the very small parathyroid glands, four or more in number, which lie close to the thyroid gland in the neck or are embedded in it, are stimulated for one reason or another to produce their hormone in excessive amounts. Calcium is then removed from the bones to such a degree that they can no longer bear the weight of the body. The individual becomes bedridden, his muscles atrophy, other consequences follow, and he dies unless he can be rescued by the removal of one or more of these very small but diseased glands. There are still other toxic hormones, some of which circulate in the body in much smaller concentration than insulin. Among them is adrenalin, or the epinephrin of our pharmacopœia. An intravenous injection of this much used and therapeutically valuable drug is fatal to animals and human beings in doses that are not so very large and furnish definite proof of its inherent toxicity. Unfortunately, also, a number of deaths have occurred from its unwise use or from overdosage. Such accidents appear to be inevitable now and then in the use of drugs in general. As I have said, this is one of the

hormones, as distinguished from insulin, of which the body appears to need under normal conditions only a very small amount in each unit of time. Its concentration in the blood, which, by the way, is sufficient for the body's ordinary needs, is minute and is represented by the astonishingly small number of 10^{-8} . It is endowed with extraordinary ability to constrict tiny blood vessels and is of the greatest service in medicine for this reason. And those who suffer from bronchial asthma obtain almost instantaneous relief from their spasmodic labored breathing after subcutaneous administration of a small amount of the hormone. But even in these patients toxic effects have been noted after a very prolonged use of the drug in too large a dose. Death has occurred from too rapid intravenous injection of one or more milligrams in the case of persons suffering from one of the thyroid diseases, and it is estimated that 10 mgm, or about one sixth of a grain, when intravenously injected, would kill a human being of average weight. I need not multiply instances of the deadly effects of excessive amounts of these peculiar principles that are so indispensable to the maintenance of health when they circulate in the body in an appropriate concentration.

It is not otherwise with the vitamins, which may be called hormones of plant origin and which are equally indispensable to the maintenance of health. One of these, irradiated ergosterol, or vitamin D, has been shown to induce very marked pathological and chemical alterations in the tissues of experimental animals, which finally lead to the death of the animals after too prolonged a medication with this principle, and there is already a considerable literature on the harmful effects of overdosage or of too prolonged an administration of this vitamin D to very young children. Fortunately, in most of these instances, the harmful symptoms disappeared after the vitamin was withdrawn. It would appear that with this drug what is called in medicine, the *dosis therapeutica* soon passes into the *dosis toxica*. Very recently a pathological state called *hypervitaminosis A* has been described as occurring in young rats that have received excessive doses of vitamin A. The disease is characterized by a faulty nutrition of the skin, inflammation of the eyes, spastic contractures of the extremities and cessation of growth, all of which pathological alterations may lead to the death of the animal. Early discontinuance of the overdosage is followed by disappearance of these symptoms and resumption of growth. It is thought that this disease is not likely to occur in human beings, as they would probably not receive such very large doses of the vitamins as were given to rats.

I have spoken to you of hormones and vitamins to show how difficult it is to define the word "poison." From the wider biological view we should not think

of poisons as being inherently more malevolent than any of the other agents or influences of our environment to which we are constantly exposed. I incline to the belief that no living cell exists whose contents or metabolites are not toxic to some other living cell; in other words, all cells are in reality cryptotoxic systems.

WHAT IS DISEASE?

Death hath a thousand doors to let out life, . . .—*Massinger: A Very Woman*.

The late Sir Clifford Allbutt, the distinguished regius professor of medicine of the University of Cambridge, has said, "Disease is the correlative of health and is incapable of a more penetrating definition." So also we find it difficult to define health in a concise sentence or two. People speak of one as being in good health, or in bad health, or that he enjoys only a moderate degree of health. Statistical determinations are not available, as far as I know, that would furnish us with a statement of what might be called a "normal state of health" for various periods of life. It is evident that there must be degrees of disease, that vary from one that is not determinable by any of our present methods of diagnosis to one that is plainly recognizable as such a departure from the normal that we can characterize it as disease. The great Claude Bernard pithily remarked: "The healthy are really invalids unaware of their illness."—(Garrison). Disease and health, then, are correlatives not clearly distinguishable from each other at certain stages. Everything that lives is constantly menaced by one external agent or another and is therefore subject to disease in varying degree, according to circumstances. The botanist, zoologist and other biologists here present will bear me out in this statement. Plant pathologists and immunologists have shown how great is the number of bacterial, virus, fungoid, algal and other infections of plants, and that in their reaction to disease plants behave in a manner not fundamentally distinct from that seen in animals. There is an essential unity in living things in all their innumerable and varied forms. The potentialities of life are illimitable in kind and degree in respect to its chemical, physical and architectural capacities.

Striking examples of the manner in which the protozoa and other lower forms of animal and plant life react to invasion by bacteria and other injurious agents are found in scientific writings before the middle of the last century. Metchnikoff, who was himself a student of the infectious diseases of the protozoa, as well as those of man, has expressed the opinion that the causes of infectious diseases might have been disclosed before the 70's and 80's of the last century, if students in that field of science had paid more

attention to the comparative physiology and pathology of the 1830's.

We may now take up the main thesis of this address, namely, that innumerable chemical substances in our environment and, to a lesser extent, abnormal products in our own bodies are the direct inciting causes of a very large number of diseases. Without attempting to give an estimate of the number of such chemical agents, we may say that many of the derivatives of the constituents of the earth's crust, a great number of chemical principles of plant and animal origin, a large percentage of the hundred thousand and more synthetic products of the chemist's skill, such as the so-called "war gases" and those that are employed in our industries, are all capable of inducing serious diseases in man and his domestic animals and, within limitations, in the various forms of plant life on which he depends for his existence. The number of such active chemical agents, to which we must add all our drugs, is very large and runs far up into the thousands.

Man has never given up the search for substances that would either cure or alleviate his ailments, and this belief has been fully justified in the course of the ages. By trial and error and by planned experimentation in more recent times valuable remedies of preventive or curative character have been discovered. From early times also and in every corner of the globe man has sought for and found so-called "stimulants"—hypnotics, inebriants, mental sedatives and hallucinating substances—that affect his psychical states. Some of these, as opium, the coca leaf, the seeds and other parts of the Solanaceae, for example, have given us sedative and other principles of the highest value in medicine. Among western nations we find beer, wines and other alcoholics, coffee, tea and tobacco in daily use as "stimulants," and people in general do not speak of them as poisons, and very properly so, when they are used in moderation, with due regard to age, state of health and other factors.

For more than a century medical scientists and biologists have occupied themselves with the action of chemical substances, to use a most inclusive term, on man and other forms of animal life and also on various kinds of plant life. The effects produced in living cells vary from barely perceptible functional or physiological and micro-structural alterations to structural alterations of such character and degree that life can no longer persist. I have long held the opinion that every reversible functional change in a cell or cell state is accompanied by a reversible, if only microphysical, alteration of structure which, in many instances, as in the autonomic nervous system, for example, can not be detected by present-day methods.

The word *reversible* is not here used in the sense of the physicist when he speaks of a *reversible process*, but only to designate a departure from the normal state of an organ where *restitutio ad integrum* finally occurs. But even in physiological and structural restitutions a price has to be paid, but this can be done because of the peculiarly favorable mechanisms involved and because the energy needed for the restitution can be supplied as needed by other structures of the body. Extreme departures from the normal can not be too often induced at short intervals without final damage to the system in which they occur.

Scientists place great emphasis on the fact that living mechanisms are endowed with the capacity to *retard* the degradation of energy that occurs in other natural processes. This retardation, as is well known, is seen at its best in the plant world. We should not, however, regard the storage of potential energy by plants and the delayed increase in entropy during their span of life as something that sharply and uniquely differentiates them from non-living systems. The physicist, C. E. Guye, of Geneva, has pointed out in his philosophical essays on physico-chemical evolution that in the system, ocean-water-vapor-atmosphere-mountain-ocean, we have, at the expense of solar energy, an arrangement in which there also occurs a retardation in the increase of entropy. The water evaporated from the ocean rises in the air, descends to mountain tops, gives rise to water courses that do mechanical work in eroding their beds and in other ways, and in due time the cycle is completed with a marked retardation of the degradation of energy. "In this way evaporation struggles against an immediate degradation of energy just like plant organisms . . . it would be wrong to attribute to the vital processes an exclusive monopoly of the struggle, if it is desired to speak of the striving against the degradation of energy."

Two great lines of investigation are always to be borne in mind in these studies of the interaction, or interplay of actions, between the agent studied and living cells. What changes have taken place in the chemical structure of the agent during its sojourn in the living thing? A very large number of substances have been studied from this point of view, and such studies come under the heading "fate of the substance in the organism." The ability of living systems of all kinds to effect the most varied and profound chemical alterations in substances that are not found in their own cells—substances that are new and strange to them, so to speak—is most extraordinary. The discoveries in this branch of pharmacology to which many specialists have devoted years of research have greatly enlarged our knowledge of the chemical mechanisms here concerned. Many agents penetrate deeply into the citadel of life, and what life does to them in a chemical way is all in the day's work and differs, as a rule, in quantitative fashion only from the usual routine of metabolism. However, there are many instances when the living organism forms, in

response to the appropriate agent, an apparently entirely new substance, which it has never before produced. Substances of this class have become of the greatest theoretical and therapeutical importance within the lifetime of the seniors among us, and are named antitoxins or immune bodies. I regret that I have not time to discuss this interesting subject further in this brief address.

The other great field of research in the interplay of actions referred to is far more difficult and more complicated than is the study of the fate of a chemical substance in the organism. It occupies itself with the functional and structural alterations induced by chemical agents taken in from the environment or formed in various cells of the organism itself. It would take me too far afield to say anything in respect to these numerous and varied functional alterations, but I shall, however, permit myself to say a few words in regard to the structural changes produced by certain doses of our chemical agents acting over a shorter or a longer period of time. Here I shall not take into consideration highly corrosive agents, such as strong acids, alkalis and other substances of inorganic nature, but will confine my remarks solely to those agents that belong to organic chemistry. Substances of this class, as I said earlier in this lecture, are the direct or proximate cause of many diseases.

A few examples only of diseases of chemical origin that may cause profound structural alterations in various tissues of the body can be cited here. Among these are the gangrenous and convulsive types of ergot poisoning, which, from the early middle ages to our own time, have destroyed thousands of lives or have permanently maimed an even larger number of people; the many forms of forage disease that so often destroy cattle, horses and sheep, and the infectious diseases of these and other domestic animals, in so far as the causative agent of these diseases is a definite chemical principle. Other examples of chemically induced disease are found among those produced in man by the numerous varieties of pathogenic microorganisms. Of the many diseases due to this large class of agents I can take into account here only such diseases of man as are caused by the bacillary forms. Many bacteriologists and pathologists now incline to the belief that, in the greater number of such infections, chemical agents play the dominant rôle in the observed symptoms and in the cellular lesions that finally occur. They believe that there are excellent reasons, based on both experimental and clinical observations, for believing that toxins are elaborated during the course of all bacterial infections, even though the exact origin and nature of these toxins is not always known. It has been demonstrated, however, that in some of the infectious dis-

eases of this class, such as tetanus, diphtheria and botulism, the bacillus in each instance serves only to produce a toxin which is responsible for the chain of events. I shall give presently a brief account of some of our experiments with the toxin of tetanus; but I wish particularly to impress upon your minds the fact that when the pathologist studies the lesions that occur in human beings and animals as the result of the above-named and many other forms of chemically induced disease, he is confronted with the same evidences of direct injury to cells, particularly those of highly specialized function, as are encountered in diseases whose etiology is less definitely established.

It was remarked in a preceding part of my address that everything that lives is constantly menaced by one external agent or another and is therefore subject to disease. It was also shown that there are autogenous sources of disease whose primarily inciting causes or etiology are not always known. Disease in its reversible early stages can not be more clearly defined until we are better informed in respect to the many chemical and physical processes and the composition and function of the many microphysical structures that are concerned with the maintenance of the ever-varying "normal state" of cells or cell states. When disease reaches that stage where, in one or more organs of the body, inclusive of the entire nervous system, functional or structural deviations from the condition called health are recognizable by the clinician and the pathologist, and especially when death ensues, we are all too ready to regard disease from our human point of view as something that a kinder nature might have ordered otherwise. In the wider biological conception disease is no more and no less malevolent or purposeful than any other of the fundamental processes or characteristics of life. Whether it has a "survival value" in the philosophy of evolution I can not say. Old age is our last disease.

I have attempted to give you in a very general but inadequate way some conception of the very large number of poisons of organic and inorganic origin that are capable of inducing disease. Only a very few of the many diseases whose cause is definitely assignable to agents of this character could be named in our brief summary. I am far from asserting that all the departures from the normal state that are due to poisons can always be differentiated from each other in all their stages as separate clinical entities. In the early stages of poisoning, as also in the later chronic period and especially when the patients are entirely unaware of the inciting cause of their illness, the physician is limited to the study of the complexes of symptoms, called syndromes, that may vary from the organic or physical to the psychopathic, or a combination of these types, and he may be quite unable

to say from the evidence at hand what poison has caused the illness or, in fact, that it is due to any poison. When, however, the symptom-complexes include one or more elements either of a functional or structural type, or a combination of the two, that are uniquely characteristic of the action of a well-known poison a more complete diagnosis can be made. In the great majority of instances of chemically induced disease the fact is easily established that one or more poisons are involved. We have also seen that, in many of the clearly recognizable infectious diseases, the efficient agent that produces the morbid state is a poison generated by the infective microorganism. We have furthermore gone so far as to believe that there exists in organized nature not a single living cell whose contents or metabolites are not toxic for some other living thing, or may not on occasion be injurious to the cell itself. I can not but believe that the subject-matter of my address, with whose central thought many of you are entirely familiar, has become, in our day, of great significance for every department of medicine as well as for many aspects of biological research.

EXPERIMENTS WITH THE TOXIN OF THE BACILLUS TETANI²

I MAY now be permitted to give a brief account of some experiments with the causative principles of the disease known as tetanus or lockjaw, a truly frightful disease of the central nervous system terminating in exhausting and fatal convulsions. It is fortunately one of the rarer diseases of man, but in time of war and on certain occasions, such as Fourth of July celebrations, it occurs more frequently. It has long afflicted the race, and historians of medicine find it described in the Hippocratic writings and other early sources of medical knowledge. Of all our domestic animals, the horse is more subject to it than we, and the loss of these animals from tetanus, more especially in tropical countries, is considerable.

Until 1884 the cause and true nature of the disease remained unknown. In that year two Italian investigators demonstrated the transmissibility of the disease to animals by injecting them with a little purulent material from a small lesion of an individual with a fatal attack of tetanus. In the years 1884-1890, bacteriologists definitely established the infective nature of the disease by isolating the causative organism in pure culture. One ordinarily speaks of this organism as the bacillus tetani, but it is known to the specialist as *Clostridium tetani*, one of the family of the bacillaceae of the general class of plant organisms known as Schizomycetes.

My associates, Dr. B. Hampil, of the Department

² This part of the address was not presented in its entirety.

of Bacteriology of the School of Hygiene and Public Health of the Johns Hopkins University, Professors F. C. Lee and W. M. Firor, of the surgical department of the Johns Hopkins Medical School, Mr. E. A. Evans, Jr., and I have been occupied for more than a year with experiments relating to certain aspects of this disease.

As there is only time for a very concise presentation, I shall summarize the main characteristics of the disease and the results of our own experiments and those of others in the following pages.

(1) That the disease may appear, the bacillus tetani or its spores must be introduced beneath the skin. Here the bacillus readily multiplies, if it meets with the proper conditions for its growth, such as association with relatively harmless pus-forming organisms as is generally the case, and a diminished tension of oxygen, or a necrosis of cells when spores only are introduced. The bacilli are not disposed throughout the body but continue to multiply for a time at the site of inoculation. Only very rarely and under special circumstances are they or their spores found elsewhere in the body.

(2) During the time of bacillary growth in the infected area a highly poisonous substance called tetanus toxin appears. This exceedingly potent, water-soluble substance is now known to be the true cause of the disease. A few bacteriologists believe that the bacillus does not itself produce a toxin, but rather a relatively harmless pro-toxin, which is transformed into the deadly toxin in culture media or at the site of infection in the presence of albumose-like substances. Nothing whatever is known of the chemical nature of the toxin. It is being constantly removed from its place of origin by lymphatic vessels and by the blood capillaries and is then distributed throughout the body by the arterial current. A boy with "lockjaw" is as truly poisoned as if he had been bitten by a rattlesnake.

(3) It is thought that this toxin of entirely unknown character finally reaches certain cells of the spinal cord and brain and by an action on them induces generalized convulsions of the most violent nature. The disease is now generally defined as clearly and solely one of the central nervous system. Certain of its symptoms, however, as the so-called local tetanus and the long-continued rigid states of certain muscles, lasting for weeks or months, that are occasionally observed in man, can not be brought into line with this concise definition of the disease. I can not enter here into a more detailed and accurate description of the course and extent of the alterations that finally affect the nervous and muscular systems.

(4) Our toxin, which is known only by its action in the animal organism, and this is true also of many other principles of its class, is of an exceedingly great

potency, excelled only by the botulinus toxin found in meats and canned vegetables infected with the botulinus bacillus (*Botulus*—a sausage). It has been found that crude precipitates of the tetanus toxin will kill a mouse weighing 15 grams in the minute quantity of 0.00000005 gram. I think it more than likely that such precipitates contain only about 10 per cent., or even less, of the poison. Assuming the sensitivity of man to be equal to that of the monkey, which again is about equal to that of the highly sensitive horse, I may hazard the calculation that it would require only 0.01 mgm of the really pure poison to kill a man weighing 150 pounds, on the supposition that my calculations have any factual basis.

(5) During the entire time the toxin is being absorbed from its place of production, and often for some days following the cessation of its production, or after the excision of the infected organ as in animal experiments, there is observed a so-called period of incubation, that is to say, a time during which there are no observable signs of illness. This time of incubation varies in human beings and in such very susceptible animals as the horse from a few to many days. When this period of incubation in human beings is not more than five or six days, the mortality is nearly 90 per cent.; when it is longer, say 10 days or many more, the mortality is still about 50 per cent. The period of incubation in the very susceptible animals is considerably reduced when larger doses of the toxin are injected into them or when, in the natural disease, a very large amount of the poison is produced in the original lesion. It is never in any case, except after the intravenous injection of immense doses, reduced to less than three to four days. In the relatively insusceptible rabbit, in which the period is about 18 hours, it can be shortened by a few hours only with enormously large doses of the toxin. French observers have found that the subcutaneous injection of thirty thousand minimal lethal doses shortened the period of incubation by four hours, while the enormous dose of ninety thousand fatal doses shortened it by two hours only. Long periods of incubation are not confined to the infectious diseases. Pharmacological literature contains many examples of this phenomenon after the injection of poisons of known chemical nature, and in the forage diseases reported from South Africa we find examples of "periods of incubation lasting as long as eighty to ninety days in horses, after the last occasion on which the poisonous plant was eaten."

(6) By what mechanism is the toxin transported to the reacting cells of the spinal cord and brain? Is it taken up by them from the blood stream, as is the case with alcohol, numerous alkaloids and many other substances? One of the most revolutionary theories ever proposed in medicine asserts that the toxin can

not be taken up from the blood stream by the reactive cells of the central nervous system, but can be absorbed by them only when (1) it is transported to them in the nerve fibrils or axons of the motor nerves, or (2) when it is transported to them in the endo- and peri-neural lymphatic vessels of these nerves. According to these theories, then, the motor nerves of the vertebrates have the hitherto unsuspected ability to transport a soluble substance from the periphery into the spinal cord. Some of the latest converts to the theory believe that the cerebrospinal fluid also receives its share of the toxin. The experiments that have been made during the past thirty years or more in support of this nerve transport theory have been so well designed and so skilfully performed by men of the highest standing, and have appeared to be so valid and irrefutable, that this unusual and exceptional mode of transport of a soluble substance has been universally accepted as an entirely satisfactory explanation of the facts of the case. Speransky and his coworkers, Ponomarew and others, have recently performed a great number of ingenious experiments with the toxin and have interpreted their findings in a manner that encouraged them to announce a third variant of the neural transport theory. They appear to be firmly convinced that the toxin and other substances that may be absorbed by the terminals of a mixed nerve, such as the sciatic, are conveyed to the cerebrospinal fluid and the substance of the cord in the tissue spaces of the nerve. I can not pause here to give an analysis of their experiments. We should have to take into consideration known facts in respect to the pressure of the cerebrospinal fluid which in the lumbar theca of a man in the recumbent position varies from 80 to 120 cm, in terms of a Locke's solution, and then inquire whether an individual with the natural disease, due to a strictly localized infection, say of the foot, would ever develop such an enormous increase of the normal tissue pressure of his foot (2 to 6 cm) as would be necessary to propel the toxin from the foot to the subarachnoid spaces of the cord—even if there were a direct and open pathway or channel to the cord. But we are not dealing in normal tissue spaces with uninterrupted continuous channels. This matter will be considered more at length in a later section of this address. We have now three variants of the neural transport theory of tetanus toxin, and it is hardly likely that the ingenuity of investigators will enable them to invent a fourth. During the world war the attention of bacteriologists, pathologists and surgeons was again directed to various aspects of tetanus. Much of the earlier experimental work on which the neural transport theory is based was repeated, with the result that some scientists now accepted carriage of the toxin both by way of axis cylinders and neural lymphatics,

while others were led to believe that the latter mode of transport suffices and is indeed the only one that can be accepted. In recent years a Japanese investigator has also published a paper in favor of this mode of transfer of the toxin to the spinal cord.

(7) It has just been stated that the theory of transport of toxin to the central nervous system by motor nerves has three variants. Let us consider first the variant that assumes that the axis cylinder is the pathway. It is evident that the toxin can not travel this pathway by diffusion, as this process is so very slow that it can not be made to explain the latent period or its variations in time. The proponents of the theory therefore assume that the toxin is propelled in the axis cylinder by a "protoplasmic streaming." As this process is also assumed to be one of slow character, it appears to account very nicely for the varying periods of incubation in small and large animals. In the smaller animals, such as mice and guinea pigs, with their shorter nerves, the time of transport would be less and would account for the shorter period of incubation in these animals.

In laboratory experiments large quantities of the toxin, often amounting to many times the average fatal dose, were injected beneath the skin of the leg of an animal, and it was found that the nerves in close proximity to the point of injection absorbed some of it. If only two to five fatal doses were injected, only the more peripheral parts of the nerve were found to contain it, while the more centrally lying parts contained none. The proof of its presence in the nerves is given by injecting the crushed nerve or an extract of it into a much smaller and more susceptible animal, such as the mouse. In any case, however, even when very large doses of the toxin are injected, the amount of toxin present in the entire nerve amounts to only a very small fraction of what was injected. The entire sciatic nerve of a full-grown cat or rabbit of average size weighs about a gram. One of the upholders of the axon transport theory found indeed that after subcutaneous injection of one minimal lethal dose into the hind leg of a rabbit not enough toxin could be recovered from the sciatic nerve to kill a small mouse, that is to say, not even

$\frac{1}{25,000}$ part of what was injected was present in the nerve. Now facts of this kind are not offered here in disproof of this variant of the transport theory now being discussed, as it may be assumed that the actual amount of toxin required by the central nervous system for the induction of convulsions must be very small indeed, and on theoretical grounds the assumed steady transportation to it of very small amounts of toxin in a unit of time might well suffice to affect the central nervous system, if this mode of transportation could be proved to exist. The occur-

rence of a very interesting and puzzling phenomenon was early noted in laboratory experiments with various small animals that had received the toxin by subcutaneous, intramuscular or intraneural injection into one of the extremities, as the hind leg, let us say. After an interval of time varying from 17 hours to four days, the muscles of the injected leg become so rigid that all freedom of movement disappears, the leg is stiffly extended and so useless that the animal walks on three legs. This spastic paralytic condition is called a local tetanus, and it is certainly a striking occurrence in that it often persists for three or four days before the muscles of the trunk or the other extremities become involved. If the injected dose is small the local tetanus finally disappears and the animal regains its normal state. This local tetanus is the corner-stone of the foundation on which the theory is built. It was assumed that in the experiments just described the toxin travels along the axis cylinders of the sciatic nerve, in the peripheral parts of which it is generally found, to the spinal cord. The motor cells of the spinal cord receiving the toxin from the nerve are thrown into a condition of hyperexcitability, lasting for some days, and in this way the cause of local tetanus is supposed to be satisfactorily explained.

The theory of carriage by nerve fibrils rests on several unproved assumptions: (1) There is no experimental evidence that there exists anything in the nature of a slow "protoplasmic streaming" or current in the axis cylinders of nerves; (2) when the toxin is found to be present in an excised piece of a nerve, such as the sciatic, there is no trustworthy evidence that an appreciable amount of it is present in the axis cylinders and that the capillaries and the endo- and perineural lymph vessels of the nerve do not contain practically all of it. It has been repeatedly shown that the lymphatic capillaries readily absorb the toxin from an injected area, and there is no sound reason for believing that the lymphatics of any nerve in the immediate vicinity of the area do not also absorb it. That "the three types of peripheral neurones, the motor, the sensory and the sympathetic, are equally able to absorb the tetanus toxin," was conclusively proved by Morax and Marie thirty years ago, who, however, nevertheless assumed that the toxin is carried upward by motor fibrils only. The findings of these authors were criticized on the ground that absorption of toxin by the neural lymphatics could not be excluded. In our day, however, carriage by the neural lymphatic seems to be the prevailing theory. In any event, how can the theory of the exclusive transport by the neurons of motor nerves be any longer upheld, in view of the observations of the French authors just cited? (3) The theory was devised to explain local tetanus and

was then applied to all cases of generalized tetanus in which no local tetanus appears. Now neither this theory nor either of its two associates can be reconciled with the following observations which were made in my laboratory by myself and my assistants a number of years ago. That lower vertebrate, the frog, is susceptible to the tetanus toxin and reacts to it with convulsions. There is no reason to believe that the proposed axis cylinder transport theory will not hold for this animal as well as for the higher vertebrates and man. Numerous experiments were made on frogs with alkaloidal convulsants and with convulsant dye stuffs. In the case of the latter convulsants their presence can be detected in the brain and spinal cord after they have exerted their action. It was very conclusively shown that all these convulsants can reach the reactive cells of the brain and cord by one pathway only, namely, by the circulating blood. Ever since those experiments were made I have entertained serious doubts in respect to the validity of the current nerve transport theory, which goes so far as to deny the possibility that the cells of the central nervous system can abstract the toxin from the circulating blood. Pochhammer has shown that local tetanus appears in the muscles of the hind leg of a rabbit that has received a subcutaneous injection of a lethal dose of toxin into the lower leg, *before* toxin can be detected in the sciatic nerve, and his findings have been fully substantiated by Sawamura, who, however, minimizes their significance, on quite inadequate grounds in our opinion. We have not yet repeated this experiment, but there can be no doubt of its correctness, and it certainly gives little support to the theory that local tetanus is due solely and entirely to an early invasion of the spinal cord by toxin by way of motor nerve fibrils. It is surely difficult to bring the fact that the peripheral parts of a nerve contain no demonstrable amount of toxin until *after* local tetanus appears, into agreement with the theory. The adherents of the theory will perhaps maintain that a small amount of the toxin was carried to the cord even though it could not be detected in the nerve.

We will not offer here a theory in respect to the true nature of local tetanus or inquire to what extent a myogenous factor is more largely involved in that form of tetanus than in the spasms of descending tetanus. I would remark, however, that the rigid, unremitting, long-continued closure of the jaws that we have observed in our experiments with horses and sheep, and that is so frequently found in man suffering from the natural disease, can hardly be distinguished from the local tetanus of a hind limb of a dog. In our opinion no such significance attaches to local tetanus as to warrant making it the basis of a theory of carriage of the toxin to the central nervous

system, either by way of the axis cylinders, the lymphatics or the tissue spaces of nerve trunks. Had not this form of tetanus been so generally observed in laboratory experiments with small animals after the injection of lethal or sub-lethal doses of toxin into them, it is very improbable that a neural transport theory would ever have been proposed. It has long been known, not only to the few opponents of the theory, but also to its numerous adherents, that local tetanus can be made the antecedent phenomenon to descending tetanus or not, as desired. In the dog or rabbit one can produce a most striking and lasting local tetanus which is later followed by spasms in other muscles, or one can so choose the site of injection and so regulate the dose of toxin that generalized descending tetanus occurs without the appearance of a definitely recognizable local tetanus. Zupnik was the first to show that even in the small animals used in laboratory experiments, local tetanus does not occur if the toxin is injected in the neighborhood of certain joints or into the tip of the toe or the dorsum of the foot. After the usual period of incubation in such experiments only *tetanus descendens* appears. Here again we have evidence that proves conclusively that local tetanus can only appear when the muscles in question are exposed to a sufficient *concentration* of the toxin and for a sufficient period of *time*. May I lay emphasis on these two conditions as indispensable to the appearance of local tetanus? There are other very striking experiments, which can not be detailed here, that indicate that local tetanus may be entirely or almost entirely averted in the dog, if the animal is made to perform hard work on a treadmill for six or more hours a day. The control animal that had received an equal dose of the toxin and was confined to its cage developed local tetanus in its most perfect form. We can not for a moment agree with Ponomarew in his interpretation of this experiment, which was first performed by him. We have repeated the experiment with great success by exercising our dogs vigorously and for longer periods of time, and in our opinion absence of local tetanus in the working dog is best explained by the assumption that the poison is rapidly removed by the capillaries and lymphatics of the injected limb in consequence of the increased circulation and the metabolic events associated with activity.

We may now consider briefly the second variant of the nerve transport theory, which states that the toxin is carried to the central nervous system by way of the endo- and perineural lymphatics. Professor Lee and his assistants have kindly performed, after consultation with us, a considerable number of experiments of different types on large dogs to test this theory. In one type of the experiments one sciatic nerve of a dog was sectioned high up in the thigh at

a convenient distance below the trochanter major, and the severed parts of the nerve were then very carefully sutured so that the continuity of the perineural sheath was restored. Naturally, all these operations were done in an aseptic manner. After the lapse of a month or more, the nerve was again exposed and one or more lethal doses of tetanus toxin, either in the form of a toxic filtrate or in the form of a solution of the dry toxin, were injected into the nerve several centimeters distal to the point of suture. At the time of the injection it was assumed that the lymph passages across the line of transection and suture had been regenerated and were capable of functioning normally. Aside from the symptoms that appeared in the injected leg, which we can not pause to describe here, it was found that generalized tetanus of the usual type occurred in the animal exactly as would have been the case had the toxin been injected into an intact sciatic nerve. It is evident that the poison could not have reached the cord by way of the degenerated axis cylinders of the sciatic, nor do we stress this point, as the advocates of the theory have always admitted that degenerated axis cylinders can not convey the poison. The advocates of the lymph transport theory will, however, find strong support for their belief in the results of this experiment. They will very naturally assert that the toxin was first transported to those centers of the spinal cord that have a functional connection with the muscles of the infected leg, and they will also hold to their belief that the large amount of the toxin that escaped from the nerve and found its way into the circulating blood was in its turn absorbed by the lymphatics of the other motor nerves of the body and transported by them to other parts of the central nervous system than those of the lower cord. We have here a fine example of the difficulties so often encountered in physiological and pharmacological experiments, namely, that the results can apparently be interpreted in at least two ways. It is a singular fact that the advocates of this theory have apparently never asked themselves the question, Where does the lymph of a nerve-trunk such as the sciatic go? The theory that we are now discussing implies that the lymph of all our peripheral nerves regularly, together with such substances as it may have taken up from the environment, finds its way to the spinal cord and the cerebrospinal fluid. Unfortunately for the advocates of this theory, a number of anatomists have finally answered the question. They have traced the course of the lymphatics of the nerve-trunks of both the fore and hind limbs. As in the case of the fore limbs, the lymphatics of the nerves of the leg accompany the arteries of the nerve and throw their contents into more deeply-lying "collectors." In all cases the lymph finally reaches lymphatic glands of the

general lymphatic system. Thus, those of the upper part of the sciatic reach deeply lying inguinal glands, and those of its lumbo-sacral trunk finally empty into the hypogastric lymph glands. In a word, we derive no evidence from the study of anatomists that the lymph of any of the peripheral nerve-trunks finds its way into the spinal cord or the cerebrospinal fluid. It may be recalled at this point that the ^{motor} nerve roots within the spinal cord have no perineural sheath and are covered only by the thin ~~neurilemma~~. It is gratifying, indeed, to have at our disposal these anatomical findings of the last few years in regard to the disposal of the lymph of nerve-trunks. It would be a remarkable fact if the central nervous system in all its parts—a system in which, according to neurologists and anatomists, there are interposed "barriers" that protect its cells from a too ready entrance into them of a great number of soluble substances—should be so accessible to all the numerous substances that can enter the lymphatics of the nerve-trunks at so many points in the body. An experiment that was made by me on frogs many years ago proves how difficult it is to introduce a powerful convulsant, such as strychnine, into the spinal canal in the case of the frog after excision of its heart and complete destruction of its four lymph hearts. A frog that has been treated in this way, and all of whose abdominal organs have been carefully removed without opening any one of the calcareous saccules that lie close to the spinal column, can be immersed in a quarter per cent. solution of nitrate of strychnine in Locke's fluid, kept oxygenated at a temperature of 12° C. for half an hour or more, without the appearance of any convulsions whatever. After removal of this "reflex-frog" from the strychnine bath and carefully washing away every trace of strychnine with cold water, it is found that it responds at once to the injection of a small amount of strychnine into the exposed spinal canal with typical strychnine convulsions. How many hours it would take for the strychnine of the bath to penetrate into the spinal canal by the slow process of diffusion, I have never determined. The experiment, however, shows very conclusively how difficult it is to introduce a rapidly acting and powerful drug into the central nervous system by any other path than by the arterial route. I may conclude this part of my address by stating my conviction that in the natural disease, tetanus, there is no carriage of the toxin to the central nervous system either by way of nerve fibrils or by way of neural lymphatics, and that the only route by which toxin can be carried to the cells of that system is the arterial pathway.

Let us now consider again the more recently proposed third variant of the neural transport theory, stating that the toxin is conveyed to the central nervous system by way of the tissue spaces of nerve trunks.

*motor
medullary
sheath*

The proponents of this theory, Speransky, Ponomarev and Wischnewsky, rest their claims on certain anatomical beliefs and on the supposed validity of deductions drawn from the results of intraneural and intramuscular injections of the toxin. We find that our experimental and critical analysis of the experiments of these investigators (which will be published in a later paper) does not support their theory in the slightest degree. Some anatomists have expressed the opinion that the tissue spaces (*Lymphräume*) of nerve-trunks, which must be differentiated from lymphatic capillaries, stand in connection with the subarachnoid and subdural spaces of the brain and spinal cord (*"im Zusammenhang stehen," Shdanow*). The tissue pressure is not of sufficient magnitude to move solutions through such narrow spaces, frequently interrupted as they are by barriers. It is freely admitted that diffusion goes on in tissue spaces of organs, as witness the lens, whose low metabolic requirements are met solely by this slow process. Diffusion can be effective only in a short space of time when it operates in structures of minute dimensions, as in the pulmonary alveolar capillaries whose wall thickness is only one thousandth of a millimeter. It would be impossible for the slow process of diffusion to do the work necessary for the maintenance of life in the four seconds of each respiratory act, were not the interposed barriers extremely thin. The minute tissue space mechanisms are energized by molecular forces only (surface energy and diffusion), and it remains to be proved that such forces can compass the astonishing feat of transporting a poison over long distances and through intervening cell barriers to the central nervous system. My associates and I have shown that, in the frog, even the foramina through which the nerves and blood vessels enter or leave the bony encasement of the spinal cord are guarded against the entrance of solutions by any other than the vascular route. Injections of suitable solutions or masses in large amounts for the purpose of outlining the entire course of arteries, veins or lymphatics are legitimate operations, since preformed pathways are utilized in experiments of this nature. But when a solution of toxin (0.1–0.3 cc and more) is injected into a living nerve-trunk, such as the sciatic of a cat or rabbit, for example, the needle of the syringe rarely if ever enters one of the veins or lymphatics of the nerve. The non-rigid, soft and distensible nerve is greatly dilated at the point of injection and the resulting internal pressure forces the solution both downward and upward in artificially formed paths. It is not permissible to believe, on the basis of such experiments, that anything of this kind occurs in the *natural* disease, even when the terminal portions of the nerve have absorbed some of the poison from the infected area. We have often

examined toxin-containing nerves, after *subcutaneous* injections of the poison, and have never observed that they differ in appearance from corresponding non-poisoned nerves. There is little likelihood of an increase in the normal tissue pressure of such nerves and little possibility here of a forcible transportation of the toxin to the central nervous system in mechanically produced pathways in the connective tissue spaces of the nerve, as is the case in nerves that have been distended by an injection. Furthermore, is it not more than likely that such of the toxin as may find its way into the tissue spaces of a nerve-trunk in proximity to the infected area, in the *natural* disease, if indeed assumption of toxin by the peripheral nerves occurs in that case, could not be carried far centrally but would be removed from these spaces by the capillaries and lymphatics? We have seen that the lymphatics of nerve-trunks finally discharge their contents into more deeply lying collectors and lymph glands that communicate with larger lymphatic trunks. Support for this opinion is found in the well-known fact that the middle and upper portions of the sciatic nerve do not contain a demonstrable amount of toxin after this has been injected subcutaneously in the region of the gastrocnemius muscle in such considerable amounts as from two to five lethal doses.

DISTRIBUTION OF TETANUS TOXIN IN THE BODY

During the past forty years and more, many investigators have shown that the toxin may be present in the spleen and liver for a short time after injection into the animal and that it persists for a longer time in the blood, lymph, lymph glands and in the peripheral parts of mixed nerves and that it also appears occasionally in the urine. It has never been found in the muscles. All this early work was of a qualitative character only, that is to say, no attempt was made to determine with any degree of accuracy how much toxin was injected and how much of it could be recovered from the blood and lymph and other parts of the body. A few quantitative experiments were made by Bieling and Gottschalk, who injected known amounts of the toxin directly into the heart of guinea pigs and then assayed the toxin content of the blood and of extracts of various organs within an hour after the injection. The results of these authors are often cited as showing that a large amount of the toxin soon disappears from the blood and that this part is either irreversibly bound or rendered inactive in various tissues and organs of the body. More than thirty years ago, Ransom, alone among early investigators, attacked the problem of the distribution of both tetanus toxin and antitoxin between the blood and lymph in a more serious manner in many experiments on dogs. After injection into the blood stream

of known and large amounts of the toxin it was found that about 26 per cent. of it had disappeared from both the lymph and blood in three hours, and after the lapse of 26 hours somewhat more than 70 per cent. of what had been injected had disappeared from the two fluids. Ransom also showed how rapidly the toxin passes into the lymph after intravenous injection and that the two fluids contain an equal amount of the toxin in equal volumes after the lapse of 26 hours. The fact that in the relatively insensitive dog the toxin disappears so rapidly from the blood and lymph is in striking contrast with our own experiments on a much more sensitive animal, the sheep.

In repeating and amplifying the earlier work of this character we have aimed primarily at the establishment of values that are easily reproducible and as accurate as our methods would permit. The quantitative data of experiments of the kind here offered are inevitably burdened with the fluctuations due to variations in the response of animals to the action of a given poison and it is therefore necessary to assemble enough data to satisfy the requirements of statistical theory and thus reduce the disturbing action of variations of unknown cause to such a degree that the conclusions thus arrived at will be valid within certain limits. We have attempted to interpret our data in accordance with this principle, but can not here give the details of our methods of assaying the amount of toxin injected by various pathways or that found to be present in the blood and lymph on subsequent days. The animals used for assaying the injected toxin and that present in the blood of the infected sheep were guinea pigs of standard age and weight, and more than a hundred of these testing animals were used for each sheep. The variation in the values obtained in these experiments is probably on the average not more than 10 per cent. and we believe that this figure represents a degree of precision far exceeding that of earlier estimations.

We have found sheep to be the most satisfactory experimental animals for these studies and have investigated the toxin content of the blood and lymph of a series of nine of these animals of approximately the same weight and after the administration of comparable doses of tetanus toxin by several routes. At the time the toxin was injected into the animals the toxicity of an aliquot portion was again carefully determined in the usual manner. Thereafter daily estimations of the toxin content of the blood were made. In this manner, a complete record of the daily variations of the toxin content of the blood of an experimental animal was obtained.

After an intravenous injection of 35.6 guinea pig minimum lethal doses of toxin per kilogram of body weight into a sheep, the toxic content of the blood

fell to 60 per cent. of the injected dose at the end of 24 hours. Forty-eight hours after the injection the blood and lymph each contained, volume for volume, equal amounts of the toxin which in the blood was still maintained at the level of 50 per cent. of the injected dose, if it can be assumed that the total amount of blood in the sheep was 6.6 per cent. of its body weight. In this animal, whose lymph had been collected on one day, the blood content in toxin dropped to less than 30 per cent. of the injected dose at the time of death. In a second animal that had not been subjected to the long operation involved in the collection of the lymph from the thoracic duct, the blood content in toxin, after intravenous injection of 53.3 guinea pig minimum lethal doses of tetanus toxin per kilogram of body weight was also estimated. One hour after the injection the blood contained 90 per cent. of the injected toxin; seven hours after the injection 60 per cent.; after 24 hours again 60 per cent. This high level was maintained up to the first appearance of trismus on the third day after the injection. Thereafter the toxin content of the blood dropped, being somewhat less than 40 per cent. at the time of death on the sixth day after injection.

When a standardized quantity of toxin amounting to from five to six lethal doses is injected at one time into a sheep, the blood and lymph content of toxin finally reach much the same level, irrespective of the site of injection, whether this be the tip of the tail, the subcutaneous tissue of a leg, the interior of a muscle or a vein. We give here two instances in which intramuscular injections were made. One of the two sheep received, by this route, 49.6 guinea pig minimum lethal doses per kilogram of body weight, while the second received 54.1 guinea pig minimum lethal doses per kilogram. The toxin content of the blood of these animals varied from 40 to 50 per cent. of the amount of toxin injected and was maintained at this high level during the three to five days that preceded the onset of symptoms. It is indeed extraordinary that the highly toxic blood can penetrate every region of the body during the several days of the incubation period without apparently manifesting any evidence of its poisonous nature. An analogous instance is that of the action of certain convulsant dyestuffs on frogs when the blood, surcharged with dyestuff, also circulates for a certain time without exhibiting any signs of toxicity. And many other examples of this kind are known, but I can not be sure at the moment that the blood content of poison during the period of incubation has actually been determined for any of them. In the experiments under consideration the toxin content of the blood dropped somewhat after the appearance of general symptoms but was still present at a concentration of 20 to 30 per cent. of the injected dose at the time of

death. With the assistance of Professor Lee we collected the lymph from the thoracic duct of one of our sheep, 36 hours after it had received an intramuscular injection of 40.7 guinea pig minimum lethal doses of tetanus toxin per kilogram. Just before the operation for the collection of lymph, the toxin content of the venous blood of the sheep was 40 per cent. of the amount injected. At this time, the lymph contains, volume for volume, an amount of toxin equal to that in the blood. Unfortunately we have no reliable data in regard to the total volume of lymph in the higher animals, and for this reason an estimate of the actual percentage of the injected toxin present in the lymph can not be made. However, it is apparent that after intramuscular injection of the tetanus toxin, as after intravenous injection, an equilibrium exists, if we may term it so, between the blood and lymph in the sense that equal volumes of both fluids contain equal amounts of the toxin. Could we assume, as was done by Ransom in his experiments on dogs, that the total amount of lymph in the entire lymphatic system, inclusive of the lymph glands, can be conservatively estimated as equal to that of the blood, we would be in a position to affirm that fully 90 per cent. of the injected toxin is present in the blood and lymph of sheep under the conditions of the experiments here described.

We have also made an experiment in which a total of 1,600 guinea pig minimum lethal doses (45.7 guinea pig lethal doses per kilogram of body weight) were injected intramuscularly into a sheep. This quantity was not injected at one time but was divided into sixteen portions, and these were injected into a limited area of a shoulder muscle at regular intervals over a period of thirty-two hours. On comparing the toxin content of the blood of this animal with that of a sheep receiving a markedly smaller dose of toxin (40.7 guinea pig minimum lethal doses per kilo) in a single intramuscular injection, we find that only 30 per cent. of the injected toxin was present in the blood during the incubation period as compared with 40 to 60 per cent. in the blood of the second animal at the same time. It is evident that the amount of toxin bound by the tissues is relatively greater when it is introduced into the body in small amounts over a period of time. The situation is somewhat more

comparable to that observed in the natural disease than when a large dose is injected at one time.

Perhaps the most striking fact that has been established by these studies is the persistence of the toxin, in high concentration and over a long period, in the vascular and lymphatic systems of the animal. That such surprisingly large amounts of the toxin can be shown to circulate in the blood and lymph up to the time of the death of an animal is indeed surprising when we recall the unanimous opinion of other workers in this field that the toxin is rapidly, and, to a very considerable degree, detoxified or bound by the animal tissues.

It will be seen from the above account of our investigations that my associates and I have come to the conclusion that the theory of carriage of tetanus toxin to the central nervous system by way of the peripheral nerves is no longer tenable. In so far as this poison reaches the central nervous system it can do so only by being brought to it by the arterial blood. The various aspects of the disease have been the subject of intensive investigation by clinicians, bacteriologists, immunologists, physiologists and pharmacologists during the past fifty years. A great body of frequently conflicting facts has been brought to light, but there is little agreement in respect to their explanation. Four different theories have been proposed to explain local tetanus and four more have been devised to show the pathways by which the toxin reaches the central nervous system. We have discussed three of the second quartet as variants of the neural transport theory. The fourth, which receives our hearty support, states that the toxin can reach the central nervous system only by way of the blood stream. In regard to the exact nature of the poison or poisons concerned, their possible alteration in the animal body and the manner in which they induce the formation of an anti-body, we are without definite knowledge. All these questions are of great significance and have an important bearing on the mode of action of bacterial toxins as a class. It will no doubt require the united efforts of many investigators for many years to find an adequate solution of these and other fundamental problems of this disease. We are continuing our work in the hope of throwing some light on one or another of these difficult questions.

